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Scientific Edition

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Investigations of the Yield and Quality of the Oils from Some Hybrid and Tetraploid Mints*,†

By A. F. SIEVERS, M. S. LOWMAN, and MABEL L. RUTTLE‡

Hybrids produced by crossing Japanese mint with *Mentha spicata*, the common wild spearmint, gave good yields of oil per acre which was low in menthol content. Hybrids of Japanese mint with *Mentha aquatica* produced oils high in menthol content but low in yield per acre. Both hybrids were more vigorous than the female parent, and one was resistant to mint rust. Two tetraploids of spearmint obtained by colchicine treatment contained only traces of oil.

IMPROVEMENT in the species and varieties of the mints grown commercially for oil production could be of much benefit to growers. Greater resistance to diseases is especially desirable because control of such diseases is in some cases impractical or too costly. Nelson (1), who has made some progress along this line, reports that oil samples obtained from some peppermint-spearmint hybrids resembled peppermint oil more than spearmint oil but that the plants look more like spearmint. Some resistance to *Verticillium* wilt of peppermint was observed. Baines (2), in attempting to develop disease-resistant peppermint, has obtained fertile peppermint by treating the common, highly sterile, black peppermint with colchicine and has crossed this with spearmint and with the Japanese mint. *Mentha arvensis* var. *piperascens* Malinvaud, the species widely grown in Japan and China as a commercial source of natural menthol, could be improved to advantage by increasing its general vigor, its capacity to survive a wide range of winter conditions, and the menthol content of its oil. Until recently when some menthol became available from Brazil our imports of this product were obtained entirely from Japan and China. A number of efforts have been made to introduce this species into this country. In California the crop was quite successful about fifteen years ago but was abandoned when menthol prices declined. In the eastern states and in the regions where peppermint (horticultural varieties of *Mentha piperita* L.) is grown commercially, the Japanese species was also tried but there the

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† Credit is due J. W. Kelly, Division of Drug and Related Plants, for assisting in the growing of the plants and the distillation of the oils.

‡ Formerly with the New York Agricultural Experiment Station, Geneva, N. Y.

crop was not entirely successful because the plant lacked general vigor and did not propagate as rapidly as desired. In 1933 the results of numerous trial plantings of this species in various sections of the country were published (3). With these general objectives in mind, extensive cytological and embryological studies of the genus *Mentha* were begun about fifteen years ago, a preliminary report of which was published in 1931 (4). These investigations were conducted at the New York Agricultural Experiment Station, Geneva, N. Y., where the crosses were made in 1929 and 1930.

As the experiments developed and the distillation of the oils from the hybrid and tetraploid forms and the examination of these oils to determine the commercial values of the new types became necessary, the cooperation of the Division of Drug and Related Plants of the Bureau of Plant Industry, Soils, and Agricultural Engineering of the U. S. Department of Agriculture was requested. It was arranged that the Division mentioned would grow the several hybrids or tetraploids that showed desirable characteristics at the Bureau's farms at Arlington, Va., and Beltsville, Md., distill the oils from them, and determine the physical and chemical properties of such oils. Special attention was to be given to the hybrids of the Japanese species with respect to their usefulness as a source of menthol.

This paper reports briefly on the nature of the oils obtained from hybrids resulting from crossing the Japanese mint with *Mentha spicata*, the common wild spearmint, and with *M. aquatica*, water mint.

Fig. 1.—*M. arvensis* var. *piperascens*. (No. 30-111-1 in Tables I and II.)

SOURCE OF MATERIAL AND DEVELOPMENT OF HYBRIDS

Varieties of the five main species of European mints, namely, *Mentha longifolia* (L.) Huds., *M. rotundifolia* (L.) Huds., *M. spicata* L., *M. arvensis* L., and *M. aquatica* L., were collected in Germany, Switzerland, and England by Miss Ruttle in 1928 and 1929. The plants were brought to the New York Agricultural Experiment Station in the fall of 1929 where crosses were made between the different species in conjunction with studies on their chromo-

Fig. 2.—Hybrid *M. arvensis* var. *piperascens* × *M. aquatica*. (No. 21-44-4 in Tables I and II.)

some number and embryology (4). Seedlings obtained from self-pollination of the parent species were grown as well as seedlings from crosses between the different species (5, 6). The selfed progenies of many of the species, in particular of *M. spicata*, were exceedingly variable even though the parent plant corresponded in considerable detail to the type specimen of the variety of the particular species. This variability included such characters as shape and hairiness on the leaf, growth habit, time of maturity, odor, and disease resistance.

Plants of the Japanese mint, *M. arvensis* var. *piperascens* (Fig. 1), were obtained from the Pharmaceutical Institute, Berlin-Dahlem. The leaves of this mint have a strong odor of menthol but the yield of menthol is much lower than that from certain other strains of Japanese mint. It grows vigorously but is highly susceptible to mint rust and the roots winter-kill rather readily in the vicinity of Geneva, N. Y. It sets a little self-pollinated seed. The progeny from self-pollination was variable in time of maturity, growth habit, size and shape of leaf, odor, and resistance to disease. Selections from its seedlings showed a more pronounced menthol odor. It crossed readily with the different species of wild mints mentioned above.

Plants obtained from the three crosses of Japanese mint × *M. longifolia*, *M. spicata*, and *M. aquatica* were all more vigorous than the female parent and had a distinct odor of menthol. For this reason some of them were selected for further tests.

Plants from the cross between Japanese mint and *M. longifolia* had an extremely vigorous root system but were very susceptible to mint rust and were consequently discarded without any examination of the oil they produced. From the cross between Japanese mint and *M. aquatica* two sister seedlings were selected for testing designated as 31-44-4 (Figs. 2 and 3) and 31-44-12 (Figs. 4 and 5). These differ in erectness, in number and shape of leaves

Fig. 3.—Flowering top of hydrid shown in Fig. 2

and spikes, and in time of maturity. Both plants have much more vigorous root systems than the Japanese parent and are in addition completely resistant to the mint rust prevalent in the locality of Geneva, N. Y. The crosses between Japanese mint and *M. spicata* yielded variable but extremely vigorous seedlings which possessed a distinct odor of menthol. Four seedlings from three different crosses were tested. Two of them designated 31-45-1 (Figs. 6 and 7) and 32-45-8 are sister seedlings from a cross with an especially fragrant *M. spicata*. A hybrid, designated 31-46-3, the *M. spicata*, parent of which was an especially erect and vigorous red-

Fig. 4.—Hybrid *M. arvensis* var. *piperascens* × *M. aquatica*. Sister seedling of plant shown in Figs. 1 and 2. (No. 31-44-12 in Tables 1 and 2.)

stemmed plant, is also very erect and vigorous like the parent, with long purplish spikes and bright red stems. The plant is quite ornamental.

All the crosses between Japanese mint and the European wild mints yielded hybrids which are so sterile that so far no seed has ever been collected from them. Commercial spearmint and commercial peppermint are also completely sterile. When commercial spearmint and a hybrid Japanese mint × *M. spicata* (designated 28-125-1 and 31-46-3, respectively), were treated with colchicine, tetraploids were obtained (7). The tetraploid shoots on both plants could be distinguished from the diploid by their larger size, the difference in the strength and quality of their odor, and by their fertility. Seed was obtained from both tetraploid plants. The progenies from this seed showed much variation and indicated that selection might possibly produce more favorable types. Further generations have not been grown.

CULTURE OF THE HYBRIDS AND DISTILLATION OF THE OILS

For a number of years small plots of the various hybrids were grown at Geneva, N. Y., to observe their general growth characters, disease resistance, and fragrance, and in 1939 and 1940 plantings of some of the more promising types were made at Arlington, Va., and Beltsville, Md., for preliminary study of the oils they produced. The plots were small, the harvesting could not in all cases be done at the right time, and some of the distillations were made of the dried herb while in other cases the fresh herb was distilled. At Geneva where no distilling equipment was available the herb was dried in barn lofts, attics, etc., not always under comparable conditions or for the same length of time and then sent to Beltsville, Md., to be distilled. For this reason and because the quantity of material distilled was so small the results, on the whole, were unsatisfactory and no significance could be attached to the yields and quality of the oils obtained. However, some of the hybrids possessed so little merit as sources of oil and from other standpoints

Fig. 5.—Flowering top of hybrid shown in Fig.4.

that they were eliminated from the larger plot plantings made in 1942 at Beltsville, Md., when the harvesting and distillation of the several lots were performed under strictly comparable conditions.

These plantings included 6 hybrids and commercial Japanese mint, commercial spearmint (*M. spicata*), a tetraploid of commercial spearmint, and a tetraploid of 31-46-3, one of the hybrids of Japanese mint × *M. spicata*. The plots were approximately 1/200 acre in area and were located on light sandy loam of rather low fertility. The rootstock was planted in rows about 3 feet apart on April 25, except that of the last-mentioned tetraploid which was not planted until July 2. The plots were kept free from weeds by light hoeing early in the season and later by hand weeding. Beginning early in August various plots were harvested when the plants reached the full blooming stage and the herb distilled in the fresh condition. All the data pertaining to the harvesting and distilling are assembled in Table I and those obtained from the examination of the oils in Table II.

DISCUSSION

The probable commercial value of the hybrids and tetraploid forms grown is determined in part by the yield of oil produced per acre and the quality of the oil. From the data obtained certain conclusions can be drawn or indications noted. The percentage and per acre yields of oils from the several mints varied greatly as will be noted from

Fig. 6.—Hybrid of *M. arvensis* var. *piperascens* × *M. spicata*. (No. 31-45-1 in Tables 1 and 2.)

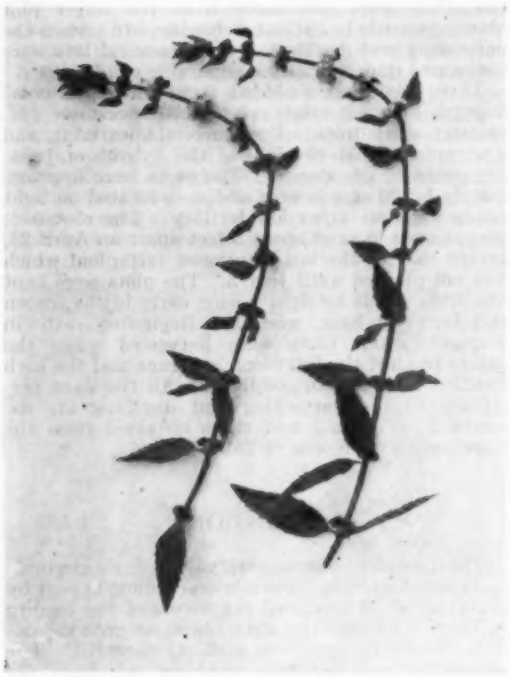

Fig. 7.—Flowering top of hybrid shown in Fig. 6.

Table I. The two sister seedlings of the hybrid Japanese mint × *M. aquatica* were comparatively poor, both in percentage and per acre yield, especially 3-44-4, which appears to be practically worthless for commercial culture. All four hybrids of Japanese mint (*M. arvensis* var. *piperascens*) × spearmint (*M. spicata*) yielded higher percentages of oil than the maternal parent, three of them being very superior in that respect. Inasmuch as all four also produced a good yield of herb the per acre yield of oil compares well with that obtained from commercial mint fields.

The oils from the two hybrids of Japanese mint × *M. aquatica* are of interest from the standpoint of quality in that they compare favorably in menthol content with the maternal Japanese mint. However, their usefulness as sources of menthol may not depend only on the menthol content. The limited experiments that could be made with the small quantities of oil available gave some indication that the menthol from the oils from the hybrids does not crystallize as readily when the oils are refrigerated as that in the oil from the Japanese mint. Moreover, since neither one gave good per acre yields under the particular conditions involved, and one of them (31-44-4) was especially poor in this respect, their value for commercial purposes cannot be determined from the data at hand. However, it has already been pointed out that they have a much more vigorous root system than the Japanese mint parent and are completely resistant to the mint rust prevalent in the Geneva, N. Y., area. Therefore, under conditions more favorable to the growth of mint than those at Beltsville, Md., these hybrids may have some value.

Only one of the four hybrids, Japanese mint × spearmint (3-46-3), contained consideralbe menthol, namely, 40.47%, but only 27.13% was present as free menthol. As a source of menthol this hybrid is much inferior to the commercial Japanese mint and commercial peppermint since the oil from the former frequently contains as much as 80% and that of the latter usually about 50% of menthol.

The two tetraploids of spearmint were poor not only in percentage yield of oil but in weight of herb produced, hence the per acre yields of oil from these were very low. The quantities of oil obtained from the small plots were insufficient for determining all the physical and chemical constants.

SUMMARY

Investigations were made of hybrids of several species of mint (Japanese mint, *Mentha arvensis* var. *piperascens*; spearmint, *M. spicata;* and water mint, *M. aquatica*) and two tetraploids, with respect to their probable commercial usefulness. These

TABLE I.—YIELD OF HERB AND OIL FROM 1/200-ACRE PLOTS OF MINTS AT PLANT INDUSTRY STATION, BELTSVILLE, MD., 1941

Plot No.		Plant Designation	Date of Harvest	Yield of Fresh Herb, Lb.	Yield of Oil		
					Weight, Gm.	Per cent	Calculated per Acre, Lb.
1	30-111-1	*M. arvensis* var. *piperascens*	8-9	61.0	51.35	0.19	19.51
2	31-44-4[a]	*M. arvensis* var. *piperascens* × *M. aquatica*	9-2	24.0	14.75	0.14	6.72
3	31-44-12[a]	*M. arvensis* var. *piperascens* × *M. aquatica*	9-2	35.0	38.00	0.24	16.80
4	31-45-1[b]	*M. arvensis* var. *piperascens* × *M. spicata* 29-132-5	8-13	47.0	68.20	0.32	30.08
5	32-45-8[b]	*M. arvensis* var. *piperascens* × *M. spicata*	8-12	46.0	74.00	0.36	33.12
6	32-48-1	*M. arvensis* var. *piperascens* × *M. spicata*	8-14	29.5	62.00	0.46	27.14
7	31-46-3	*M. arvensis* var. *piperascens* × *M. spicata* 28-45-3	8-11	52.0	52.65	0.22	21.00
8	28-125-1	*M. spicata* (commercial spearmint)	8-8	15.0	32.55	0.48	14.40
9	40-125-1	Tetraploid of commercial spearmint	8-15	15.0	3.20	0.05	1.40
10	40-46-3	Tetraploid of 31-46-3	10-9	11.0[c]	3.24	0.07	1.54

[a] Sister seedlings of different types due to variability of parental stock.
[b] Sister seedlings from a cross with an especially fragrant *M. spicata*.
[c] This plot was not planted until July 2 which may account to some extent for the low yield of herb.

TABLE II.—PHYSICAL AND CHEMICAL PROPERTIES OF OILS DISTILLED FROM 1/200-ACRE PLOTS OF MINTS AT THE PLANT INDUSTRY STATION, BELTSVILLE, MD.

Plot No.	Plant Designation	Specific Gravity, 25° C.	Index of Refraction, 25° C.	Angle of Rotation, 100 Mm., 20° C.	Percentage of Menthol		
					Free	Combined	Total
1	30-111-1 *M. arvensis* var. *piperascens*	0.8931	1.4590	−36.25°	62.34	3.59	65.93
2	31-44-4[a] *M. arvensis* var. *piperascens* × *M. aquatica*	0.9035	1.4615	[c]	53.22	4.38	57.60
3	31-44-12[a] *M. arvensis* var. *piperascens* × *M. aquatica*	0.8924	1.4615	−39.40°	56.46	4.38	60.84
4	31-45-1[b] *M. arvensis* var. *piperascens* × *M. spicata* 29-132-5	0.8881	1.4535	−25.15°	3.97	5.79	9.76
5	32-45-8[b] *M. arvensis* var. *piperascens* × *M. spicata*	0.8883	1.4520	−20.75°	2.85	2.43	5.28
6	32-48-1 *M. arvensis* var. *piperascens* × *M. spicata*	0.8884	1.4536	−24.85°	3.34	3.98	7.32
7	31-46-3 *M. arvensis* var. *piperascens* × *M. spicata* 28-45-3	0.9165	1.4737	−50.70°	27.13	13.34	40.47
8	28-125-1 *M. spicata* (commercial spearmint)	0.9271	1.4883	−57.00°	...	...	...
9	40-125-1 Tetraploid of commercial spearmint	[c]	1.4847	[c]	...	...	...
10	40-46-3 Tetraploid of 31-46-3	[c]	1.4658	[c]	...	...	...

[a], [b] See Table I.
[c] Insufficient quantity of oil for determination.

were developed by the junior author in conjunction with studies on their chromosome number and embryology. In connection with the crosses of the Japanese mint it was the purpose primarily to develop hybrids of high menthol content that possessed greater vigor and resistance to the more serious diseases that cause damage in commercial mint fields. These hybrids and the tetraploids were grown in 1/200-acre plots to determine their general growth habits and provide material for distillation to determine the percentage of oil present and the quality of the oil.

The two hybrids of Japanese mint and water mint (*M. aquatica*) were more vigorous than the female parent and produced oils with 57.60 and 60.84 per cent of menthol. However, the calculated yields of oil per acre were relatively low in one case and very poor in the other. Under more favorable cultural conditions both yield and quality of oil may be somewhat better. These hybrids are completely resistant to the mint rust prevalent in the regions where they have been grown. This quality and their greater vigor could make them useful as material for further breeding and selection.

The four hybrids of Japanese mint and spearmint were also found to be more vigorous than the female parent and gave good indicated yields of oil per acre but only one of the oils contained as much as 40 per cent of total menthol. These hybrids are therefore of doubtful value as sources of menthol when grown under the conditions of this experiment.

The two tetraploids obtained by colchicine treatment included in the plot tests, one of commercial spearmint and the other of a cross Japanese mint, *M. spicata*, were extremely low in oil content (0.05 and 0.07 per cent, respectively) and appear to have no value except perhaps for further breeding work.

REFERENCES

(1) Nelson, Ray, "Present Status of Wilt-Resistant Hybrid Mints and Plans for Their Further Development," Report of Michigan Muck Farmers Assoc., pp. 38–40, 1943.
(2) Baines, R. C., "Breeding Disease Resistant Mints," in Report Purdue Agricultural Experiment Station, p. 73, 1943.
(3) Sievers, A. F., and Lowman, M. S., "Commercial Possibilities of Japanese Mint in the United States as a Source of Natural Menthol," *Tech. Bull. No.* 378, *U. S. Dept. of Agr.*, 1933.
(4) Ruttle, Mabel L., "Cytological and Embryological Studies on the Genus Mentha," *Gartenbauwissenschaft*, 4 [5], 429–68(1931).
(5) Ruttle, Mabel L., *Proc. Intern. Cong. Genetics, 6th Cong.*, 2, 311–12(1932).
(6) Ruttle, Mabel L., *The Herbarist*, 4, 17–29(1938).
(7) *Ibid.*, 5, 20–27(1939).

The Gravimetric Determination of Aluminum in Pharmaceutical Preparations*

By F. J. BANDELIN

A method for the gravimetric determination of aluminum in pharmaceutical preparations is given. The method is dependent upon the gradual evolution of ammonia from urea in boiling acid solution containing succinic acid. Aluminum is quantitatively precipitated as the basic succinate which is filtered off and ignited to Al_2O_3. This method offers advantages over the conventional hydroxide method in that the precipitate obtained is dense, granular, and easily and rapidly filtered. The maximum *p*H obtainable is 4.6, thereby eliminating the possibility of excess alkalinity. Data are given which indicate that the succinate method gives more accurate and uniform results than the hydroxide method.

THE determination of aluminum by the conventional hydroxide precipitation method has a number of disadvantages. The flocculent precipitate of aluminum hydroxide formed by the addition of ammonium hydroxide to a soluble aluminum salt is gelatinous and bulky, making filtration, and washing of the precipitate, time-consuming and difficult. Because of its translucent nature, it is difficult to see the aluminum hydroxide adhering to the glassware used for handling it; thus some of it may be overlooked and lost. It clings tenaciously to utensils with which it comes in contact, making quantitative transfer to a crucible a questionable procedure. Furthermore, the amphoteric aluminum hydroxide is slightly soluble in excess ammonium hydroxide even in the presence of ammonium chloride added for the common ion effect, and it is appreciably soluble at *p*H values over 10.0 (1). An excess of this reagent, consequently, will give low results in determinations. The possibility of adsorption or occlusion of foreign materials by the aluminum hydroxide introduces a possibility of error which cannot be overlooked, while improper washing of the precipitate in the crucible may lead to the formation of a soluble hydrosol.

A method offering considerable advantages over the hydroxide precipitation method has been described by Willard and Tang (2, 3) who demonstrated that aluminum may be quantitatively precipitated with urea in the presence of sulfate and organic anions such as oxalate, succinate, benzoate, and phthalate to give dense, granular precipitates which are much less bulky than aluminum hydroxide. The method depends upon the hydrolysis of urea in acid solution to form ammonia slowly and cause a gradual and uniform increase in *p*H resulting in a final *p*H value of 4.2 to 4.6. The aluminum is precipitated upon heating as the basic salt of the anion present. Aluminum may also be determined by precipitation as the basic acetate (4, 5, 6), but the method yields a somewhat gelatinous precipitate which resembles aluminum hydroxide and is not granular as are the precipitates obtained in the presence of the above anions.

A study of the determination of aluminum in the official pharmaceutical preparations by the urea method and by the official hydroxide method indicated that determination as the basic succinate is more rapid and expedient. A comparison of these

TABLE I

Aluminum Hydroxide Gel U. S. P. XII, 5-Gm. Sample Gm. of Al_2O_3 Found		Solution Aluminum Chloride N. F. VII, 5-Cc. Sample Gm. of Al_2O_3 Found	
Succinate Method	Hydroxide Method	Succinate Method	Hydroxide Method
0.2041	0.2032	0.2636	0.2627
0.2038	0.2029	0.2634	0.2626
0.2038	0.2021	0.2628	0.2621
0.2036	0.2018	0.2628	0.2631
0.2037	0.2031	0.2628	0.2618
0.2034	0.2031	0.2631	0.2619
0.2038	0.2036	0.2637	0.2622
0.2031	0.2029	0.2633	0.2618
0.2037	0.2030	0.2634	0.2621
0.2039	0.2016	0.2631	0.2621
0.2040	0.2021	0.2637	0.2630
0.2041	0.2019	0.2633	0.2619
0.2040	0.2036	0.3636	0.2622
0.2035	0.2035	0.2636	0.2630
0.2039	0.2035	0.2634	0.2627
0.2035	0.2018	0.2629	0.2627
0.2040	0.2035	0.2634	0.2624
0.2039	0.2034	0.2636	0.2623
0.2033	0.2038	0.2633	0.2621
0.2039	0.2034	0.2635	0.2615

* Received Jan. 19, 1945, from the Laboratories of Flint, Eaton and Company, Decatur, Ill.

methods and their relative accuracy and applicability to the U. S. P. and N. F. preparations was undertaken.

EXPERIMENTAL

A quantity of preparation equivalent to approximately 0.2 Gm. of Al_2O_3 was taken for analysis. In the case of Aluminum Acetate Solution, N. F. VII, a 10-cc. sample was used. Solution of Aluminum Subacetate required a 15-cc. sample instead of the 5-cc. sample specified in the official procedure (7). Aluminum Hydroxide Gel, U. S. P. XII was first dissolved in hydrochloric acid in preparation for determination by the succinate method.

The sample, in an 800-cc. beaker, is diluted to about 200 cc. with distilled water. Ammonium Hydroxide T. S. is added until a slight turbidity forms. This turbidity is cleared by the dropwise addition of 10% hydrochloric acid and then two or three drops are added in excess. Five grams of succinic acid (analytical reagent) dissolved in 100 cc. of water is then added. This is followed by the addition of 10 Gm. of ammonium chloride (analytical reagent) and 4 Gm. of urea. The volume of the solution is adjusted to 400 cc. with water and boiled gently for ninety minutes after the solution becomes turbid. This usually occurs about twenty to thirty minutes after boiling begins. Turbidity begins at a *p*H where methyl orange changes color. This indicator may therefore be advantageously employed to note this change in *p*H of the solution. The mixture is allowed to cool to room temperature, filtered through a tared Gooch crucible and the beaker rinsed with six 10-cc. portions of 1% succinic acid solution made neutral to methyl red with ammonium hydroxide. These rinsings are used to wash the precipitate in the crucible which, after washing, is ignited to constant weight at 1000° C. Ignition at this temperature for one hour is sufficient to convert the aluminum hydroxide to a nonhygroscopic Al_2O_3 (Corundum) which is cooled in a desiccator and weighed.

TABLE II

Solution Aluminum Acetate N. F. VII, 15-Cc. Sample Gm. of Al_2O_3 Found		Solution Aluminum Subacetate N. F. VII, 10-Cc. Sample Gm. of Al_2O_3 Found	
Succinate Method	Hydroxide Method	Succinate Method	Hydroxide Method
0.1890	0.1891	0.2370	0.2353
0.1894	0.1879	0.2372	0.2355
0.1890	0.1877	0.2368	0.2360
0.1888	0.1882	0.2371	0.2369
0.1888	0.1879	0.2371	0.2355
0.1885	0.1875	0.2370	0.2353
0.1891	0.1881	0.2371	0.2351
0.1892	0.1887	0.2367	0.2349
0.1890	0.1879	0.2371	0.2355
0.1893	0.1879	0.2370	0.2360
0.1893	0.1876	0.2372	0.2363
0.1890	0.1883	0.2370	0.2369
0.1894	0.1873	0.2371	0.2358
0.1888	0.1876	0.2370	0.2369
0.1888	0.1878	0.2372	0.2368
0.1887	0.1886	0.2369	0.2363
0.1892	0.1883	0.2370	0.2357
0.1890	0.1874	0.2371	0.2368
0.1889	0.1877	0.2370	0.2365
0.1887	0.1873	0.2367	0.2366

Determinations for comparison were carried out on the U. S. P. XII and N. F. VII preparations by the official procedures.

A special analyzed grade of aluminum metal containing 99.87% aluminum[1] was used as a standard. An accurately weighed quantity of this metal was dissolved in a small quantity of hydrochloric acid and diluted with distilled water so that a 10-cc. aliquot contained the equivalent of 0.2 Gm. of Al_2O_3.

A series of 20 determinations by each method was carried out on all preparations and on the standard. Samples of preparations were obtained from the same batch or container to assure uniformity. Tables I, II, and III give the results obtained by both methods.

TABLE III

Aluminum Standard Metal. Samples taken equivalent to 0.2 Gm. Al_2O_3[a]	
Succinate Method	Hydroxide Method
0.1997	0.1999
0.1995	0.1981
0.1996	0.1979
0.1999	0.1988
0.1998	0.1992
0.1996	0.1990
0.1995	0.1992
0.2001	0.1983
0.2002	0.1988
0.2000	0.1981
0.1997	0.1991
0.1996	0.1982
0.1995	0.1989
0.2001	0.1993
0.2001	0.1991
0.2002	0.1995
0.1995	0.1992
0.1996	0.1994
0.1997	0.1996
0.2001	0.1990

[a] Corrected for the impurities in the aluminum metal.

TABLE IV

Preparation	Succinate Method Mean, Gm.	Succinate Method Standard Deviation Mg.	Hydroxide Method Mean, Gm.	Hydroxide Method Standard Deviation, Mg.
Aluminum Hydroxide Gel, U. S. P. XII	0.2037	0.2	0.2029	0.7
Aluminum Chloride Solution, N. F. VII	0.2633	0.3	0.2623	0.4
Aluminum Acetate Solution, N. F. VII	0.1889	0.2	0.1879	0.5
Aluminum Subacetate Solution, N. F. VII	0.2370	0.1	0.2360	0.6
Aluminum Metal	0.1998	0.2	0.1889	0.5

The standard deviation from the mean weight of Al_2O_3 obtained by both methods for each of the prep-

[1] Obtained from the Aluminum Company of America.

arations and aluminum metal standard in Tables I, II, and III was calculated by the formula:

$$S.D. = \sqrt{\frac{\Sigma d^2}{n}}$$

where Σd^2 is the sum of the squares of the deviations from the arithmetic mean, and n is the number of determinations.

It is apparent from Table IV that the mean of the determinations by the succinate method is higher in every case than that obtained by the hydroxide method. This is significant in the case of the aluminum standard where it is apparent that the succinate method gives more accurate values. Also, the standard deviation from the mean is considerably smaller for all preparations by the succinate method than by the hydroxide method. This indicates greater uniformity of results by the succinate method.

SUMMARY AND CONCLUSIONS

The gravimetric determination of aluminum in pharmaceutical preparations by the basic succinate method offers a number of advantages over the hydroxide method.

The method is dependent upon the gradual evolution of ammonia from urea in boiling acid solution resulting in a maximum *p*H of 4.6, precluding the possibility of excess alkalinity. The basic aluminum succinate obtained by this reaction is dense and easily filtered and washed. It does not adsorb other materials to the extent that aluminum hydroxide does. Because of its granular nature, it is easily transferred from beaker to crucible.

The data obtained indicate that the succinnate method gives more accurate and uniform results than the hydroxide method.

REFERENCES

(1) "Scott's Standard Methods of Chemical Analysis," ed. 5, D. Van Nostrand Co., Inc., New York, N. Y., 1939, p. 11.
(2) Willard, H. H., and Tang, N. K., *J. Am. Chem. Soc.*, **59**, 1190(1937).
(3) Willard, H. H., and Tang, N. K., *Ind. Eng. Chem., Anal. Ed.*, **9**, 357(1937).
(4) Funk, W., *Z. anal. Chem.*, **45**, 181 (1906).
(5) Kling, A., Lassieur, A., and Lassieur, Mme. A., *Compt. rend.*, **178**, 1551 (1924).
(6) Mittasch, A., *Z. anal. Chem.*, **42**, 492 (1903).
(7) "The National Formulary," Seventh edition, Mack Printing Co., Easton, Pa., 1942, pp. 234, 236.
(8) "Pharmacopœia of the United States," Twelfth revision, Mack Printing Company, Easton, Pa., 1942, p. 198.

An Emetic Assay for Quinacrine Hydrochloride in Pigeons*

By CHARLES R. THOMPSON and HAROLD W. WERNER

A method is described for evaluating the emetic activity of quinacrine and quinacrine derivatives following oral administration of the compounds to pigeons. The assay method was designed to detect large differences and the data were not treated statistically. Results, however, which indicated a fair degree of sensitivity are presented.

TESTS for quinacrine hydrochloride in the United States Pharmacopœia do not include biological evaluations; nevertheless, a variety of biological methods are commonly employed to supplement chemical tests for determining the suitability of quinacrine for clinical use. The bioassay procedures used include acute and chronic toxicity determinations, tests of liver function, and evaluations of emetic action.

Certain inadequacies of present emetic assay methods prompted an investigation into the possibility of developing an emetic assay employing pigeons. Such a method should be useful in distinguishing between various batches of quinacrine and in the investigation of new derivatives of the quinacrine type.

Pigeons have been employed by a number of investigators for evaluating emetic activity. In previous assays (1–4) the drugs have been administered intravenously. In the work with quinacrine and derivatives it seemed desirable to administer compounds orally. Early experiments, therefore, were directed toward the determination of conditions satisfactory for this route of administration. Effects of volume and concentration, point of administration, time of feeding, and number of animals per dose level were investigated. While the results of this investigation apply specifically to the assay of quinacrine com-

* Received March 26, 1945, from the Research Laboratories of The William S. Merrell Company, Cincinnati, Ohio.

pounds, it is likely that the conditions outlined here will prove important in developing assay methods for other substances administered orally.

EXPERIMENTAL

Methods and Results.—Satisfactory results were obtained with pigeons of either sex, weighing 250–450 Gm. In preliminary experiments the birds were divided into three or four dosage groups of eight animals each. In later experiments three groups of ten animals each were employed. The pigeons were starved for approximately twenty-four hours before drug administration. The substance to be assayed was administered in aqueous solution in a constant volume of 0.02 ml. per Gm. of body weight. The doses, calculated in mg./Kg. of body weight, were introduced directly into the crop with a hypodermic syringe to which a 3-inch length of a No. 10 Fr. catheter had been attached. Animals were placed in individual cages and observed for a period of four hours. Retching movements or emesis were considered as a positive response.

The assay technique was determined with a single sample of quinacrine standard (5676U2), and it was further tested with other quinacrine samples and quinacrine derivatives. The *E. D.*$_{50}$ (50% emetic dose) was determined in each assay by inspection of the curve obtained by plotting the log of the dose against the probit of response (Bliss, 5). Points in these graphs, excepting those for higher dose levels, fell on a straight line. The emetic responses, at higher dose levels in individual assays (Table I) and in the standard curve (Fig. 1) frequently were low. This may have resulted from a local anesthetic action of quinacrine as it was found that higher concentrations produced corneal anesthesia in rabbits.

TABLE I.—EMETIC ASSAYS FOR TWO SAMPLES OF QUINACRINE AND ONE QUINACRINE DERIVATIVE

Preparation	Assay No.	Dose, Mg./Kg.	Emesis, %	*E. D.*$_{50}$, Mg./Kg.
Quinacrine, 9150U	1	50	10	89
		75	40	
		100	50	
	2	60	10	93
		80	30	
		120	80	
Quinacrine, A1052	1	60	30	69
		80	70	
		120	70	
	2	60	0	96
		80	40	
		120	60	
Quinacrine derivative, 192R-92	1	80	30	112
		120	50	
		170	80	
	2	80	50	80
		120	90	
		170	90	

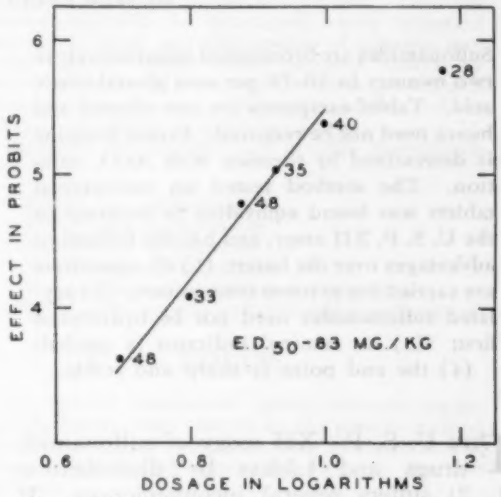

Fig. 1.—Combined results of eight assays on a standard quinacrine sample number 5676U2. Figures refer to number of pigeons on each point.

Even though some variation was observed at the higher dose levels, the method appears satisfactory. In the present investigation interest centered primarily on developing a method to detect large differences, and data have not been treated statistically. However, some measure of accuracy of the method can be obtained by noting the agreement between various assays on identical samples. The mean *E. D.*$_{50}$ in eight assays of quinacrine, 5676U2, was 83 mg./Kg., and the values ranged from 67 to 100 mg./Kg. Assays on other quinacrine samples and a quinacrine derivative indicate a similar sensitivity for the method. The *E. D.*$_{50}$'s obtained in two assays of quinacrine, 915OU, were 89 and 93 mg./Kg. and in two assays of quinacrine, A1502, were 69 and 96 mg./Kg. Assays of a quinacrine derivative, 192R-92, gave *E. D.*$_{50}$ values of 80 and 112 mg./Kg.

No indications of increased sensitivity or tolerance to quinacrine were observed in animals used once a week for a period of six to ten weeks.

REFERENCES

(1) Christensen, B. V., and Gramling, L. G., THIS JOURNAL, **26**, 32(1937).
(2) Christensen, B. V., and McLean, A. P., *ibid.*, **28**, 74 (1939).
(3) Hanzlik, P. J., and Shoemaker, H. A., *Proc. Soc. Exptl. Biol. Med.*, **23**, 298(1926).
(4) Hanzlik, P. J., *J. Pharmacol.*, **35**, 363(1929).
(5) Bliss, C. I., *Quart. J. Year Book Pharm.*, **11**, 192(1938).

Determination of Sulfonamides in Tablets*

By HERTSELL S. CONWAY†

Sulfonamides are brominated quantitatively in two minutes in 50–70 per cent glacial acetic acid. Tablet excipients are not affected and hence need not be removed. Excess bromine is determined by titration with As_2O_3 solution. The method tested on commercial tablets was found equivalent in accuracy to the U. S. P. XII assay, and has the following advantages over the latter: (1) all operations are carried out at room temperature; (2) acylated sulfonamides need not be hydrolyzed first; (3) no external indicator is needed; (4) the end point is sharp and stable.

THE U. S. P. XII assay of sulfonamide drugs and tablets by diazotization (1, 2) suffers several inconveniences. It requires an outside indicator. It requires a fairly narrow temperature range, for if the solution is too cold, diazotization is unduly slow; and if it is too warm or if the titration is too rapid, nitrous acid may be lost by volatilization. Sulfadiazine can be readily dissolved out of tablet mixtures by hydrochloric acid only by warming of the steam bath. Succinylsulfathiazole requires two hours' preliminary hydrolysis in order to liberate the aromatic amino group. The rate of diazotization close to the end point is so slow that a titration is considered complete only when the end point is reproducible after an interval of one minute. The U. S. P. XII instructions on this last point are not consistent, since the one-minute wait is specified for pure compounds, but not for tablets (1). Similarly, in the First Bound Supplement of U. S. P. XII, the one-minute wait is specified for all sulfonamides and tablets thereof, except succinylsulfathiazole tablets (2).

Bromometric assay (3–8) is rapid, accurate, and convenient for pure sulfonamides and for ointments (9). Hitherto it has not been successfully applied to tablets (3, 4) because the usual excipients react with aqueous bromine and cannot be removed easily. Wells (10) assayed tablets by removing acid-insoluble excipients by filtration, hydrolyzing the sulfonamide linkage, and then brominating for an hour; but this method takes well over two hours.

We have found that in a medium of 50–70 per cent glacial acetic acid the oxidation of excipients is negligible, while sulfonamides are still brominated quantitatively in two minutes.

The excess bromine is determined by back titration with arsenious oxide solution until the characteristic yellow bromine color is discharged. Potentiometric titrations indicated that the visible change corresponds to the true end point. The standard method of adding excess arsenious oxide and finally back titrating with bromate-bromide (11) was found unsatisfactory. Under the conditions of the assay, the recommended bromometric indicators such as methyl orange, methyl red, and Bordeaux were not destroyed until a considerable excess of bromine was present, thus yielding high results.

Iodometric determination of excess bromine is also satisfactory for sulfanilamide, sulfathiazole, sulfadiazine, and sulfaguanidine, but no stable end point could be obtained in the thiosulfate titration in the presence of brominated sulfamerazine or brominated succinylsulfathiazole. The reason for this instability is now under investigation.

EXPERIMENTAL

Reagents.—0.1 *N* arsenious oxide (11); 0.2 *N* bromate-bromide, containing 6 Gm. of potassium bromate and 30 Gm. of potassium bromide per liter, and standardized against the arsenious oxide solution (11); 2% sodium hydroxide.

Procedure.—An accurately weighed sample of powdered tablets containing 0.25–0.35 Gm. of sulfanilamide, sulfathiazole, sulfadiazine, or sulfamerazine, or 0.35–0.45 Gm. of sulfaguanidine, or 1.0–1.4 Gm. of succinylsulfathiazole is placed in a glass-stoppered flask. Twenty milliliters of 2% NaOH is added, and the mixture is shaken vigorously to dissolve the sulfonamide. Now a measured volume of 0.2 *N* bromate-bromide is added (just sufficient to provide a few milliliters in excess) followed by 80 ml. of glacial acetic acid and 5 ml. of concentrated HCl. The flask is immediately stoppered, shaken vigorously for 30 seconds to redissolve any precipitated sulfonamide, and allowed to stand one and one-half minutes longer. Finally the solution is titrated with 0.1 *N* arsenious oxide until the bromine color is discharged.

The 2% NaOH is especially necessary for dissolving sulfadiazine and sulfamerazine, but may be

* Received Dec. 7, 1944, from the Research Laboratory, Lambert Pharmacal Company, St. Louis, Mo.

† Appreciation is expressed to Lederle Laboratories, Inc., for pure sulfathiazole and sulfaguanidine; to Sharp & Dohme, Inc., for pure succinylsulfathiazole; to the above firms, Parke, Davis & Company, and Eli Lilly and Company for information on tablet excipients. The encouragement of Dr. R. R. Read, Chief Chemist of Lambert Pharmacal Company, is gratefully acknowledged.

TABLE I.—ASSAY OF SULFONAMIDE TABLETS

	Manufacturer	Assay %: Bromination	Assay %: U. S. P. XII
Sulfanilamide	A	80.3	79.5
		80.3	80.5
		81.7	80.8
		av. 80.8	av. 80.3
	B	81.1	81.5
		81.3	81.7
		....	81.8
		av. 81.2	av. 81.7
	C	82.5	82.3
		82.9	81.3
		83.1	82.3
		82.4	....
		av. 82.7	av. 82.0
Sulfathiazole	B	80.5	80.6
		81.3	80.9
		81.5	....
		av. 81.1	av. 80.8
	C	81.6	81.2
		82.6	81.9
		80.7	81.1
		80.0	....
		av. 81.2	av. 81.4
	D	81.6	80.9
		80.9	80.8
		80.8	....
		av. 81.1	av. 80.8
	E	82.9	82.0
		80.8	80.5
		82.0	....
		82.4	....
		av. 82.2	av. 81.3
Sulfadiazine	A	67.8	66.2
		67.2	66.5
		67.8	67.8
		av. 67.6	av. 66.8
	B	80.6	81.8
		81.1	81.4
		80.2	81.0
		80.6	....
		av. 80.6	av. 81.4
	D	81.6	82.5
		80.8	82.1
		80.8	81.0
		av. 81.1	av 81.9
Sulfamerazine	B	82.1	81.4
		81.6	81.4
		82.1	82.0
		82.3	....
		av. 82.0	av. 81.6
Sulfaguanidine	B	81.0	80.7
		80.5	80.8
		81.1	81.2
		81.8	....
		av. 81.1	av. 80.9
	E	83.4	83.4
		82.5	83.6
		83.5	84.0
		83.4	84.4
		av. 83.2	av. 83.9
Succinylsulfathiazole	F	77.5	77.8
		78.3	77.7
		78.2	78.0
		av. 78.0	av. 77.8

omitted from the analysis of sulfanilamide and sulfathiazole, and is of course useless for dissolving sulfaguanidine. In the latter three cases the acetic acid may be added to the sample first; then the bromate-bromide is introduced with constant swirling to avoid any local excess of aqueous bromine. (Acetic acid releases bromine slowly by itself, but not at all if sodium acetate is present and mineral acid is absent.) HCl is then added, and the analysis concluded as described above.

Each net milliliter of 0.2 *N* bromide corresponds to:

0.008610 Gm. sulfanilamide
0.008511 Gm. sulfathiazole
0.008342 Gm. sulfadiazine
0.008810 Gm. sulfamerazine
0.01161 Gm. sulfaguanidine monohydrate
0.03734 Gm. succinylsulfathiazole monohydrate

RESULTS

Commercial sulfonamide tablets, representing six manufacturers, were assayed by the present method and by the U. S. P. method. The tabulated results indicate satisfactory agreement between the two methods.

DISCUSSION

In the proposed bromometric assay, all operations are carried out at room temperature; acylated sulfonamides need not be hydrolyzed first; no external indicator is needed; and the end point is sharp and stable.

SUMMARY

The bromometric assay of sulfonamide tablets in 50 to 70 per cent glacial acetic acid is equivalent in accuracy and superior in convenience to the U. S. P. XII assay by diazotization.

REFERENCES

(1) "United States Pharmacopœia," Twelfth revision, Mack Printing Co., Easton, Pa., 1942, pp. 439–40, 443, 487–88.
(2) "United States Pharmacopœia," Twelfth revision, 1st Bound Supplement, Mack Printing Co., Easton, Pa., pp. 49–55, 63–65.
(3) Hoshall, E. M., *J. Assoc. Official Agr. Chem.*, **22**, 748 (1939).
(4) Conway, H. S., *ibid.*, **25**, 791(1942).
(5) Sol'ts, F. M., *Ukrain. Gosudarst. Inst. Eksptl. Farm.* (Kharkov), *Konsul'tats. Materialy* **1940**, No. 1, 31–2; through *C. A.*, **36**, 3000(1942); the abstract has "0.1 *N* KBr," which should read "0.1 *N* $KBrO_3$" instead.
(6) Chiarino, J. C., and Ferrer, I., Jr., *Ph.* **1941**, No. 2, 10–16; through *C. A.* **36**, 1566(1942).
(7) Dehusses, J., *Mitt. Lebensm. Hyg.*, **32**, 242–49(1941); through *C. A.* **37**, 3464(1943).
(8) Fishburn, A. G., and Smith, M. J., *Pharm. J.*, **150**, 76(1943).
(9) Conway, H. S., THIS JOURNAL, **32**, 169(1943).
(10) Wells, E. H., *J. Assoc. Official Agr. Chem.*, **25**, 747 (1942).
(11) Kolthoff, I. M., and Sandell, E. B., "Textbook of Quantitative Inorganic Analysis," revised ed., The Macmillan Company, New York, 1943, pp. 585–87, 622–23.

The Determination of Nitrogen in Theobromine by the Kjeldahl Method*

By C. W. BELL†

A brief review of the Kjeldahl method for the determination of nitrogen is presented together with its limitations. A method is outlined for the determination of the nitrogen in theobromine whereby the use of a correction factor is eliminated and results can be obtained with a relative error of less than 0.5%. This method is based upon a preliminary digestion of the sample on a water bath and then proceeding with the Kjeldahl–Gunning–Arnold modification.

REMARKABLE progress has been made in the determination of organic nitrogen by the classical Kjeldahl method during the sixty-two years since this method was first published (1). Since then its use has become widespread and it is regarded as a very important tool of analytical chemistry. Fundamentally, it is a wet oxidation whereby the nitrogenous substance is decomposed, in the presence of concentrated sulfuric acid and suitable catalyzers, to yield ammonium sulfate quantitatively. After liberation with concentrated alkali, the free ammonia thus formed is determined by any of the several methods available.

The Kjeldahl method is universally used in the routine determination of nitrogen since the results can be obtained quickly and with practically the same order of accuracy as obtained by the longer and more tedious Dumas or Absolute Method. However, as in any analytical method, the Kjeldahl process is subject to certain limitations and serious errors will be made if such facts are not recognized.

Many natural products and pure organic nitrogenous compounds have their nitrogen in a basic form which is easily split off during the digestion process to form ammonium sulfate. However, there are some nitrogenous compounds in which the nitrogen is not in a basic form; that is, the nitrogen is contained in a nitro, nitroso, azo, or some other similar group. These compounds are decidedly refractory and yield their nitrogen by the Kjeldahl method with difficulty (2–5). Compounds containing nitrogen in the ring, such as pyridine, nicotinic acid, quinoline, pyrazolons, and the xanthines are also very stubborn and are not completely decomposed into ammonium sulfate unless certain modifications are resorted to (6–9). Theobromine, a member of the xanthine group, is a heterocyclic compound containing imid groups in the purine ring and therefore is somewhat refractory. Low results are usually obtained unless a correction factor is employed or some modification of the original Kjeldahl method is used. The Gunning–Arnold modification (7, 10) is often used with a fair degree of accuracy in ordinary routine work. However, it is not applicable for very precise work, probably because of loss due to sublimation during the preliminary period of digestion since it is an established fact that theobromine sublimes at 290–295° C. (11, 12). This sublimation is encouraged and intensified by the suction used for removing fumes in most Kjeldahl setups. Incidentally, it is also impossible to determine the nitrogen in certain other aromatic nitrogenous compounds by the usual Kjeldahl method because of sublimation (13).

For very precise work and to eliminate the need of a correction factor the author for several years has been using a preliminary digestion period in conjunction with the Gunning–Arnold modification. This digestion is accomplished by means of very low heat preferably on a water bath and eliminates the error that would be introduced by sublimation of the sample before the conversion to ammonium sulfate is completed.

EXPERIMENTAL

For the purpose of this investigation two series of determinations were run on chemically pure theobromine; that is, theobromine conforming to the standards and tests of the N. N. R. (14) and each sample containing 0.05% or less of moisture. One series was run by the regular Kjeldahl–Gunning–Arnold method without a preliminary digestion and the second series was run by employing the preliminary digestion on a water bath in conjunction with the above-mentioned method. The complete procedure is as follows:

Place an accurately weighed sample of 0.15–0.18 Gm. of theobromine in a 500-cc. Kjeldahl flask and

* Received Jan. 24, 1945, from the Control Laboratory, E. Bilhuber, Inc., Orange, N. J.
† Appreciation is expressed to Mr. R. O. Hauck for his helpful suggestions in carrying out this work.

TABLE I—COMPARISON OF RESULTS WITH AND WITHOUT PRELIMINARY DIGESTION BEFORE THE REGULAR KJELDAHL-GUNNING-ARNOLD MODIFICATION

Sample No.	Percentage of Nitrogen (Theory, 31.11%) With Preliminary Digestion	Without Preliminary Digestion
1[a]	31.04	30.03
	31.02	30.24
	31.06	30.12
	31.00	29.97
Av.	31.03	30.09
2[b]	30.99	30.26
	31.15	30.68
	31.14	30.48
	30.99	30.45
Av.	31.07	30.47

[a] 0.05% of moisture in sample.
[b] 0.02% of moisture in sample.

add 15–18 Gm. of anhydrous sodium sulfate together with 1 Gm. of copper sulfate and 25 cc. of fuming sulfuric acid. Heat the Kjeldahl flask with contents on a boiling water bath for thirty minutes, occasionally shaking it to make sure that the digestion mixture comes in direct contact with all of the sample. Remove the flask from the water bath and place in an inclined position in the usual manner. Heat the mixture gently until frothing ceases, making sure that the temperature is maintained below the boiling point of the acid. Gradually increase the heat until the acid boils briskly and digest for about two hours after the mixture has turned green, or until oxidation is complete. After cooling, dilute with about 100 cc. of water and add a few pieces of granulated zinc to prevent bumping. Add sufficient concentrated sodium hydroxide solution to make the reaction strongly alkaline, pouring it down the side of the flask so that it does not immediately mix with the acid solution. Connect the flask to a condenser by the use of a connecting bulb and place the tip of the condenser below the surface of 50.00 cc. of 0.1 *N* sulfuric acid contained in the receiver; carefully mix the contents of the flask by shaking and distill until all of the ammonia has passed over into the measured quantity of the standard acid. Titrate the excess of 0.1 *N* sulfuric acid with 0.1 *N* sodium hydroxide solution using methyl red as indicator. Each cc. of 0.1 *N* sulfuric acid is equivalent to 0.001401 Gm. of nitrogen.

RESULTS

Table I shows a series of results with and without the preliminary period of digestion. These results are typical of those obtained over a period of several years and indicate that the nitrogen in theobromine can be determined with a relative error of less than 0.5% by using the preliminary period of digestion on a water bath whereas without this preliminary period of digestion the results are usually 2.0–3.0% low.

REFERENCES

(1) Kjeldahl, J., *Z. anal. chem.*, **22**, 366(1883).
(2) Rieman, W., and Neuss, J., "Quantitative Analysis," McGraw-Hill Book Co., New York, 1937, p. 135.
(3) Phelps, I. K., and Daudt, H. W., *J. Assoc. Official Agr. Chem.*, **3**, 306(1920).
(4) *Ibid.*, **4**, 72(1920).
(5) Hillebrand, W. F., and Lundell, G. F., "Applied Inorganic Analysis," John Wiley & Sons, Inc., New York, 1929, pp. 640–41.
(6) Fisher, Harry L., "Laboratory Manual of Organic Chemistry," ed. 3, John Wiley & Sons, Inc., New York, 1934, p. 298.
(7) "United States Pharmacopœia," Twelfth revision, Mack Printing Co., Easton, Pa., 1942, p. 598.
(8) Schmidt, Julius, "A Text-Book of Organic Chemistry," ed. 2, D. Van Nostrand Co., Inc., New York, 1932, p. 7.
(9) Mann, F. G., and Saunders, B. C., "Practical Organic Chemistry," Longmans, Green & Co., Ltd., London, 1938, p. 297.
(10) "Methods of Analysis," ed. 5, Association of Official Agricultural Chemists, Washington, D. C., 1940, p. 26.
(11) Beilstein, F., "Handbuch der Organischen Chemie," J. Springer, Berlin, Vol. 26, 1937, p. 458.
(12) "Merck's Index," ed. 5, Merck & Co., Inc., Rahway, N. J., 1940, p. 550.
(13) Margosches, B. M., & Kristen, W., *Z. ges Schiess-Sprengstoffw.*, **18**, 73–76(1923); through *C. A.*, **17**, 3656(1923).
(14) "New and Nonofficial Remedies," American Medical Association, Chicago, Ill., 1943, pp. 353–54.

CORRECTION—June, 1945, page 174, "Cascara Sagrada Berries, an Adulterant of Buckthorn Berries, N. F. VII," by Clare Olin Ewing and Albin Stitt. The second sentence in the second paragraph should read as follows: "We have been obliged to reject several shipments and purchasing samples that consisted largely of the dried ripe fruit of *Rhamnus Purshiana* de Candolle."

The Volatile Oil from *Lophanthus anisatus* Benth.*

By ERNEST H. POLAK†,‡ and R. M. HIXON

This investigation was undertaken with the purpose of expanding the limited information available on the volatile oil of *Lophanthus anisatus* Benth. or "anise hyssop." Variations in oils obtained from plants grown and processed under different conditions were studied. The presence of the following oil constituents was demonstrated: Methylchavicol (main constituent), *d*-limonene, probably α-pinene, and a mono- or bicyclic sesquiterpene. Upon standing the oil formed various methylchavicol polymers from which could be isolated two dimers, isoanethole and probably "non-sublimable" metanethole, and two unidentified ethers. It is suggested that the dimers of methylchavicol might be of interest in the study of synthetic estrogenic compounds.

THE mint plants or *labiatae* are excellent bee-feeding plants. *Lophanthus anisatus* Benth., popularly called "anise hyssop" or "fragrant giant hyssop," is a native prairie perennial of Iowa. Although the anise hyssop never disappeared completely from Iowa, experimental plantings as a nectar source were made with plants from a Canadian source in 1939 by Mr. Frank Pellet at Atlantic, Iowa.

The tufted growth and the sturdy, well-ramified roots of the anise hyssop give promise that this plant may be used also as a means to check soil erosion. Presumably plantings at the Federal Soil Erosion Nursery at Ames were made for this purpose and probably from plants gathered in Emmet County about 1932.

Information regarding the volatile oil obtained from anise hyssop is very limited. Schimmel & Co. (1) reported the specific gravity and optical rotation of a sample supplied to them in 1894 by Professor Kremers of Madison, Wis. Later in 1913 the same company (2) reported some physical and chemical constants of an oil originating from the botanical garden at Marseille, France. The brown-green oil which had been obtained in a 0.11 per cent yield contained about 80 per cent methylchavicol. A steam-distilled oil from a closely related plant, *Lophanthus rugosis* Fisb. et Mey., native to East Asia was reported by De Vilmorin and Levallois (3) to contain 86 per cent methylchavicol and probably some *d*-limonene and other hydrocarbons.

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The anise hyssop should not be confused with the "giant hyssop," *Agastache pallidiflora* (Heller) Rydberg, or "hyssop," *Hyssopus officinalis* L., which also produce volatile oils but of different composition. Apart from the botanical name *Lophanthus anisatus* Benth. referred to in the chemical literature, the anise hyssop is also known as *Agastache foeniculum* (Pursh) Kuntze, *Agastache anethiodora* (Nutt.) Britton, *Hyssopus anetheodora* Nutt. Fras. Cat., *Hyssopus anisatus* Nutt., *Vleckia anisata* Raf. Fl. Tell., and *Vleckia anethiodora* Greene. Mem. Torr. Bot. Club.

COLLECTION OF THE VOLATILE OIL

Variations in the volatile oil of anise hyssop plants grown at Ames and at Atlantic are summarized in Table I. In general, the plants were cut while in bloom, dried, chopped, and steam distilled in 3- to 5-Kg. batches. Most of the oil came over in the first ten minutes and none after sixty to seventy minutes. Two to three gallons of condensate was obtained for each 5 Kg. of dried plant material. Cohobation of the cloudy condensation water gave no further recovery of oil. The steam-distilled oil was separated from the water, dried over sodium sulfate, and finally filtered.

The physical and chemical constants of the oils grown at both sources indicate that the oil obtained from plants grown at Ames is superior in regard to yield, as well as quality in terms of methylchavicol content, when compared with the oil obtained from plants grown at Atlantic. A superficial comparison between the plants grown at both sources did not reveal any fundamental differences although the Ames plants had larger, better developed flower tops, more leaves and thinner stems than the Atlantic plants. It remains to be determined whether these anomalies are due to differences of genetic character or to soil, climate, or cultivation.[1]

The yield of terpene compounds in a typical Ames batch (No. 2) was 0.05%, that of an Atlantic batch (No. 3) was 0.16%, as compared with, respectively, 0.75% and 0.22% for the over-all yields of oil.

[1] Sievers (4), van Fleet (5), and Rabak (6) have discussed extensively the effects of various cultivating conditions on two other *labiatae*, the peppermint and the spearmint plant.

Such a difference indicates that the two oils have an unlike composition.

The yield of oil also varies with different plant parts. The leaves and flowers of batch No. 4, which had been separated mechanically from the stems before grinding, gave 0.14% of oil while the stems gave only 0.024% of oil. Similarly, the plants of batch No. 5, which had not been cut during the summer and were harvested late in September, contained heavy woody stems and gave 0.42% of oil. This compares with a 0.76% yield for batch No. 6 harvested at the same time and consisting of abundantly leafed, thin-stemmed plants which had grown to a height of one to two feet after having been cut in July to furnish material for a batch No. 2 (0.75% yield). Batch No. 1, which had not been dried sufficiently before grinding, became moldy under storage. In order to determine whether the low yield (0.08%) and the low methoxy value (3.35%) of the resulting oil were due to the mold or due to the fact that the plants originated from Atlantic, a 200-Gm. sample from the combined batches No. 5 and No. 6 was intentionally moistened and allowed to mold for seven weeks. The molded plants were then steam distilled and gave 0.03% yield of an oil having a methoxy content of 2.3%, corresponding to 11% of methylchavicol. Two hundred grams of nonmolded plant material from the same batch gave 0.6% yield of oil having a methoxy content of 18.6% corresponding to 89% of methylchavicol. The experiment indicates that storage of the dried plants over a period of two and one-half months did not alter the yield or quality of oil but that both are reduced by mold growth.

erratic values obtained for the optical activity might be due to racemization of the optical active terpene and sesquiterpene parts by polymerization.

Various changes observed in the oil after storage are summarized in Table II. Batches Nos. 1, 2, 3, 4, 5 and 6 had been stored in bottles which were filled only three-quarters full. Batch B had been stored in two identical completely filled bottles. One of these gave 10.4% of residue upon distillation, the other 20.4%. Possibly factors other than light and air are involved in the polymerization process. All oils had been stored in a dark place since methylchavicol is known to undergo changes by the action of daylight (7) as well as by air (8). The addition of an inhibitor like hydroquinone might reduce polymerization.

Nitrogen analyses of the plant residue left after steam distillation gave an average protein content of 14.1, 8.94, and 8.75%. Legume roughage has a protein content of about 12%. A preliminary test conducted by the Animal Husbandry Subsection of the Agricultural Experiment Station indicated that cattle will consume the plant residue.

CHEMISTRY OF THE OIL[1]

The chemical examination of oils from various batches was limited by the small quantities of oil available.

In the oil obtained from the planting at Ames could be identified 5–10% *d*-limonene and 82–93% methylchavicol. Upon standing for eight months,

TABLE I.—DATA ON ANISE HYSSOP AND OIL DISTILLED FROM IT

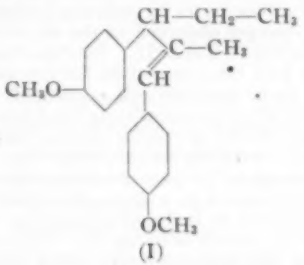
Batch No.	A[a]	B[a]	1	2	3	4	5	6
Origin	Ames	Ames	Atlantic	Ames	Atlantic	Atlantic	Ames	Ames
Month and year harvested	7-42	7/8-42	6-43	7-43	8-43	9-43	9-43	9-43
Weight dried plants, Kg.	3.86	8.63	15.28	21.35	13.10	12.40	10.60 4.30	3.60 2.10
Weight oil, Gm.	Not dried 10	40	12	159	28.5	6	18	16
Per cent yield on dry basis	0.26	0.46	0.079	0.75	0.22	0.048	0.42	0.76
Remarks	Wet		Plants molded					
Specific gravity $\frac{25°}{25°}$	....	0.9592	0.9072	0.9606	0.8924	0.8951	0.9536	0.9538
Refractive index ($n_D^{25°}$)	....	1.5200	1.4955	1.5200	1.4956	1.4998	1.4771	1.4760
Optical rotation ($\alpha_D^{25°}$) 10 cm. tube	....	−5.73°	+7° 38′	+3° 37′	−3° 24′	−33° 28′	−10° 38′	−7° 18′
Per cent RO as methoxy[c]	....	14.0	3.35	19.4	5.73	5.59	19.1	18.5
Per cent ROR′ as methylchavicol[d]	....	17.1[b]	16	93	27	27	91	88

[a] Batches A and B distilled and measured by Dr. Brimhall.
[b] Determined by Polak on same oil after standing 1 yr.
[c] Per cent RO as methoxy: Per cent methoxy by Zeisel micro method.
[d] Per cent ROR′ as methylchavicol = per cent methylchavicol calculated from per cent OCH_3 assuming that all methoxy is derived from methylchavicol.

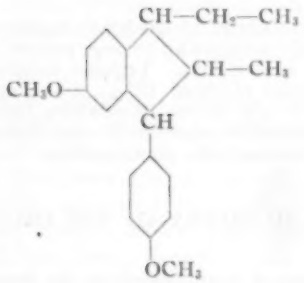
As is apparent from Table I, only the specific gravity can be used as a physical criterion for oil quality since the values obtained for refractive index and optical rotation change with time. The physical constants must be determined on the fresh oil since, on standing, the methylchavicol polymerizes slowly to compounds having a high refractive index. The 11–25% of the available methylchavicol polymerized. From the polymerized residue could be isolated isoanethole (I), a trace of what is probably metanethole (II), and two unidentified derivatives of methyl-

[1] The recorded melting points were determined on a Fisher-Johns hot stage. They were uncorrected.

chavicol of melting point 53–55° and 163–164°, respectively.



(I)



(II)

From the oil obtained from plants grown at Atlantic could be isolated 13–32% of methylchavicol, 20–24% of *d*-limonene, a trace of probably α-pinene, and a mono- or bicyclic sesquiterpene which could be dehydrogenated to cadalene but remained unidentified otherwise.

Phenols or aldehydes could not be detected in oils from either source.

The percentage of methylchavicol obtained from the oils by distillation agreed generally within 4–5% of the methylchavicol content calculated from the methoxy value. Since no other phenolic ethers were isolated it can be assumed that the methoxy content of the oil reflects fairly accurately the methylchavicol content of the oil.

Isoanethole and metanethole had only been synthesized by dimerization of anethole (9). Whether anethole is an intermediate for the formation of methanethole and isoanethole from methylchavicol is not known. In this respect it is significant that neither anethole nor anisaldehyde, one of the former's oxidation products, was isolated from the anise hyssop oil. Glichitch (10) was unable to synthesize any metanethole from methylchavicol by a procedure giving a 5% yield (9) from anethole. In recent abstracts it is reported that van der Zanden, de Vries, and Dijkstra (11) obtained metanethole only by treating methylchavicol with boron fluoride. In this laboratory it was found that metanethole can be prepared in a 2.2% yield from methylchavicol by boiling it with 34% sulfuric acid according to the corresponding directions for anethole (9) which gives a 24% yield. This method is only a modification of one of the earlier procedures developed by Perrenoud (12) who treated anethole with anhydrous zinc chloride. The latter method gave only traces of metanethole from methylchavicol. Campbell, Dodds, and Lawson (13) called attention to the structural similarities between anethole dimers and α,β-diethylstilbestrol which is one of the most potent synthetic substances exhibiting estrogenic activity. The separation and purification of the constituents of the oil were affected by careful vacuum fractionations.

Methylchavicol.—Methylchavicol isolated from the various batches was fractionated once over sodium. (Constants: b. p. 215°, $n^{17}_{D}{}^{.5°}$ 1.5230.) The following derivatives were prepared: Anethole, m. p. 21°, $n^{25°}_{D}$ 1.5590 (14); anisic acid, m. p. 184°; and homoanisic acid, m. p. 84° (15).

***d*-Limonene.**—The fraction of b. p. 175° obtained by repeated fractionation of the lower boiling parts of batch No. 3 and further purified by distilling two times over sodium showed the following constants: $n^{20°}_{D}$ 1.4716, $d^{25°}_{25°}$ 0.856, $[\alpha]^{25°}_{D}$ + 108°. The following derivatives were prepared: The tetrabromide m. p. 100–102° C. (16), the dihydrochloride m. p. 48–49° (17), and the β-nitrosochloride, needles from chloroform, m. p. 100–102° (18).

α-Pinene.—Batch No. 3 contained 0.5 Gm. of a low-boiling fraction which after having been distilled over sodium had the following constants: b. p. 156°, $n^{25°}_{D}$ 1.4670. A nitrosochloride prepared

TABLE II.—SOME CHANGES OBSERVED UPON STORAGE OF ANISE HYSSOP OIL

	Batch No.	Before Storage	After 8 Mo. Storage	After 12 Mo. Storage
$n^{20°}_{D}$	B	1.5200	....	1.5239
	3	1.4956	1.4964	
	4	1.4998	1.5143	
	5	1.4771	1.5320	
	6	1.4760	1.5210	
$d^{25°}_{25°}$	B	0.9592	....	0.9969
$\alpha^{27°}_{D}$	B	−5.73	....	−18
Per cent of residue[a]	B		....	10.4–20.4
	1		38.6	
	2		19.7	
	3		12.2	
	4		56.6	
	5 and 6		11.2	
Remarks	B, 1, 2, 5, 6	....	Odor and color same as before storage	
	3	....	Slight acetic acid odor	
	4	Light green color	Clear; very green color; very viscous; turpentine odor	

[a] As residue was considered that part of the oil which could not be distilled at 200° C. liquid temperature and 1-mm. pressure.

by the procedure of Thurber and Thielke (19) gave white tables melting at 101–103° after four recrystallizations from methanol.

Sesquiterpene.—A fraction of b. p. about 250°, $n_D^{25°}$ 1.4965, $d_{25°}^{25°}$ 0.907 was obtained from batch No. 3 after repeated fractionations and a final distillation over sodium. These constants indicate the presence of a bicyclic or monocyclic sesquiterpene. Passing hydrogen chloride gas through a well-cooled ether solution gave, upon evaporation of the ether and dilution with cold methanol, flocculent oily crystals which could not be purified by any of the usual ways. Dehydrogenation of 0.7 Gm. of the suspected sesquiterpene fraction with 0.33 Gm. of sulfur, following the directions of Ruzicka (20), gave upon vacuum distillation of the reaction products about 100 mg. of an aromatic-smelling oil, which after freeing from sulfur compounds by distillation over sodium and treating with picric acid, gave the picrate as dark orange-colored needles melting at 110.5–112.5°. Cadalene picrate melts at 114–115° (20).

Isoanethole and Metanethole.—Three fractions of b. p. about 200°/0–1 mm. had been collected from batch No. 3. The first two fractions, totaling 1.2 Gm., showed $n_D^{25°}$ 1.5835, $d_{25°}^{25°}$ 1.12. Isoanethole has b. p. 192–193°/0.1 mm., $n_D^{25°}$ 1.5863 (9). The product was further recognized as isoanethole by oxidation with potassium permanganate in acetone, giving anisic acid, m. p. 184°, and α-*p*-methoxyphenyl-*n*-propyl methyl ketone [semicarbazone m. p. 189° (13)] in the proportions recorded by Goodall and Haworth (21).

From the third fraction long needles separated which were expected to be metanethole, the usual companion of isoanethole (9). Crystals obtained after four recrystallizations from a mixture of ether and Skelly "D" sublimed between 110° and 120° at atmospheric pressure and started melting at 134° in a sealed tube. Perrenoud (12) recorded a sublimation point between 115° and 120° and a melting point of 132° for metanethole without giving any further details as to how these constants were obtained. Baker and Enderby (9), who identified the structure and reviewed the literature of metanethole, recorded a melting point of 134° but ignored the sublimation point mentioned by Perrenoud (12) and Glichitch's (10) general statement that metanethole is "sublimable." Metanethole prepared by the action of 49% sulfuric acid on either methylchavicol or anethole, following Baker and Enderby's (9) directions for anethole, was nonsublimable and had the m. p. 134°. It was further characterized by demethylation to metanethol, m. p. 156° (9) and nitration to dinitromethanethole, m. p. 191° (9). A mixed melting point of the sublimable and the nonsublimable metanethole gave one degree depression after the mixture had been melted, cooled, and pulverized three times. Further investigation seems necessary to explain the above observations.

Substances of M. P. 53–55° and 163–164°.—Distillation of the residue obtained from batch No. 2 in an all-glass apparatus gave a low-boiling fraction of b. p. 110°/0–1 mm., $n_D^{25°}$ 1.5847, and a high boiling semisolid fraction of b. p. 200°/0–1 mm.

Half of the low-boiling fraction crystallized after a week in the icebox. The flaky crystals were separated from the liquid part, washed three times with cold ether, recrystallized twice from ether, and finally recrystallized from a mixture of Skelly "D" and 10% ether; m. p. 53–55°. The same product was obtained from the residues of batches No. 5 and No. 6. The compound did not react with bromine in carbon tetrachloride, was insoluble in water, and reduced a dilute alkaline permanganate solution to the green manganate stage. Molecular weight by micro Rast method 179–189; methoxy content 20.5–21.0%. Methylchavicol has a methoxy content of 20.9% and a molecular weight of 148.

The high-boiling semisolid fraction was washed four times with cold ethanol in which the liquid part was soluble. It was then recrystallized twice from ethanol and once from a mixture of Skelly "D" and 10% chloroform; m. p. 163–164°. The compound did not discolor a solution of bromine in carbon tetrachloride appreciably and an aqueous potassium hydroxide suspension slowly reduced an alkaline potassium permanganate solution to the green manganate stage. (Molecular weight by micro Rast method 343–345; methoxy content 19.9–20.3%.) The compound might be a dimer of methylchavicol which would have a methoxy content 20.9% and a molecular weight of 296. Van Romburgh (7) and van der Zanden (22) reported a dimer of methylchavicol which melted at 166° but remained otherwise unidentified.

SUMMARY

1. Volatile oils obtained from anise hyssop grown at Ames contained *d*-limonene and methylchavicol. Oils obtained from plants grown at Atlantic contained *d*-limonene, methylchavicol, a sesquiterpene, and probably a trace of α-pinene.

2. Prolonged storage of oils obtained from Ames plants resulted in the formation of polymerized methylchavicol residues from which could be isolated isoanethole, probably sublimable metanethole, and two unidentified ethers.

3. Nonsublimable metanethole was synthesized from methylchavicol.

REFERENCES

(1) *Ber. Schimmel & Co. Akt. Ges.*, April 1898, p. 58.
(2) *Ibid.*, October 1913, p. 17.
(3) De Vilmorin, P., and Levallois, F., *Bull. soc. chim.*, [4] **15,** 342(1914).
(4) Sievers, A. F., *U. S. Dept. Agr., Bull.*, 1555 (1929).
(5) Van Fleet, W., *U. S. Dept. Agr., Bull.*, 694 (1917).
(6 Rabak, F., *U. S. Dept. Agr., Bull.* 454 (1916).
(7) Van Romburgh, P., *Verhandel. Akad. Wetenschappen Amsterdam, Afdeel. Natuurkunde*, **17,** 16(1909).
(8) Gildemeister, E., and Hoffmann, Fr., "Die Ätherischen Öle," ed. 3, Verlag der Schimmel & Co. Akt. Ges., Miltitz, 1931, Vol. 3, p. 1001.
(9) Baker, W., and Enderby, J., *J. Chem. Soc.*, **1940,** 1094.
(10) Glichitch, L. S., *Bull. soc. chim.*, **35,** 1160(1924).
(11) Van der Zanden, J. M., de Vries, M. G., and Dijkstra, R. H., *Rec. trav. chim.*, **61,** 280(1942); through *C. A.*, **38,** 2330(1944).
(12) Perrenoud, P., *Ann.*, **187,** 68(1877).
(13) Campbell, N. R., Dodds, E. C., and Lawson, W., *Proc. Roy. Soc. (London)*, **128B,** 253(1940).
(14) Gildemeister, E., and Hoffmann, Fr., "Die Ätherischen Öle," ed. 3, Verlag der Schimmel & Co. Akt. Ges., Miltitz, 1928, Vol. 1, p. 603.
(15) Bertram, J., and Walbaum, H., *Arch. Pharm.*, **235,** 179–82(1897).
(16) Huntress, E. H., and Mulliken, S. P., "Identification of Pure Organic Compounds," ed. 1, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 575.
(17) Simonsen, J. L., "The Terpenes," ed. 1, University Press, Cambridge, 1931, Vol. 1, p. 133.
(18) Wallach, O., *Ann.*, **252,** 112(1889).
(19) Thurber, F. H., and Thielke, R. C., *J. Am. Chem. Soc.*, **53,** 1030(1931).
(20) Ruzicka, L., *Fortschr. Chem. Physik physik. Chem.*, **19A,** [5], 57 pp. (1928).
(21) Goodall, G. D., and Haworth, R. D., *J. Chem. Soc.* **1930,** 2482.
(22) Van der Zanden, J. M., *Rec. trav. chim.*, **57,** 233(1938).

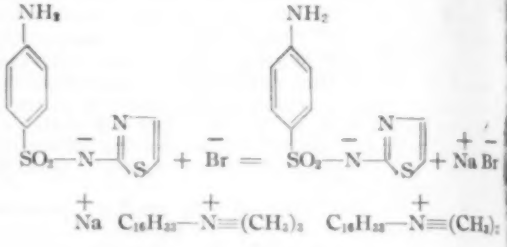
Quaternary Ammonium Salts of Sulfanilamide Derivatives*

By RICHARD H. BARRY and BRUNO PUETZER

Quaternary ammonium salts of sulfanilamide derivatives have been prepared by reacting the sodium or silver salts of the sulfonamides with quaternary ammonium halides. In this manner a number of salts have been prepared and a brief summary of their physical and chemical properties is given.

SULFANILAMIDE and its derivatives are extensively used in the treatment of skin infections, wounds, and burns. Since these compounds have comparatively low bactericidal activity and low solubility, it seemed desirable to investigate the possibility of their combination with the soluble, highly bactericidal, and surface-active quaternary ammonium bases. It was thought that compounds containing both sulfonamide and quaternary ammonium residues might be useful in nasal therapy and in other chemotherapeutic applications. Domagk (1) reported the high antiseptic action of long-chain alkyl quaternary compounds, Shelton (2) and others (3, 4) have observed optimum bactericidal activity in compounds possessing one cetyl radical ($C_{16}H_{33}$), particularly cetyltrimethylammonium bromide and cetylpyridinium chloride. Mixtures containing various amounts of sulfa drugs and quaternary ammonium salts are used in some commercial products.

The acidic properties of some of the sulfanilamide derivatives suggested the possibility of salt formation with these quaternary bases. We have prepared such salts by reacting the sodium or silver salts of the sulfanilamide compounds with quaternary ammonium halides. The following equation shows the formation of cetyltrimethylammonium sulfathiazole from cetyltrimethylammonium bromide and sodium sulfathiazole:



EXPERIMENTAL

The preparation of cetyltrimethylammonium sulfathiazole illustrates the methods used in the preparation of the quaternary ammonium salts of the sulfa drugs listed in Table I and will be described in detail.

Cetyltrimethylammonium bromide, 3.64 Gm. (0.01 mole) dissolved in a minimum of distilled water was added to 3.0 Gm. (0.01 mole) sodium sulfathiazole sesquihydrate dissolved in 25 ml. water. The slightly alkaline reaction of the latter quickly disappeared and precipitation of cetyltrimethylammonium sulfathiazole resulted. The product was recrystallized from hot water and occurred as a

TABLE I.—QUATERNARY SALTS OF SULFANILAMIDE DERIVATIVES

Compound	Appearance	M. P. (Uncorr.), °C.	% Sulfur[a] Calcd.	% Sulfur[a] Found
Cetyltrimethylammonium sulfathiazole-hydrate[b]	Pearly, white	Sinters 107 Melts 141	11.50	11.61
Cetylpyridinium sulfathiazole-hydrate[c]	Deep yellow	Sinters 95 Melts 119	11.10	11.12
Cetyltrimethylammonium sulfadiazine-dihydrate	White	143	5.62	5.65
Cetyltrimethylammonium sulfamerazine-dihydrate	White	126	5.49	5.51
Cetylpyridinium sulfapyridine-dihydrate	Slightly yellow	109–110	5.45	5.45
Laurylpyridinium sulfathiazole	Yellow	76–80	12.76	12.70

[a] Sulfur analyses by Mr. Glenn B. Hess.
[b] Calculated for H_2O, 3.23%; found 3.19%.
[c] Calculated for H_2O, 3.12%; found 3.12%.

* Received March 23, 1945, from the Vick Chemical Co. Research Laboratories, Flushing, N. Y.

hydrate. This salt was also prepared by warming 3.64 Gm. (0.01 mole) cetyltrimethylammonium bromide and 3.9 Gm. (0.011 mole) silver sulfathiazole in 50 ml. ethanol for twenty minutes. After filtering the silver bromide, most of the alcohol was evaporated, 100 ml. of water was added, and the solution allowed to crystallize. Other alkali, alkali earth, or metal salts of sulfa drugs might serve the same purpose.

The compounds listed in Table I were made using the methods given above. All are crystalline salts and usually occur as hydrates. They vary in their solubility in water, are soluble in dilute mineral acids and the water-miscible solvents. The melting points of the products are not sharp; sintering is usually observed before complete melting. They give red colorations when diazotized and coupled with either β-naphthol or R-salt. Addition of dilute silver nitrate or copper sulfate to a hydroalcoholic solution of the quaternary ammonium sulfanilamide derivatives precipitates the metal salt of the sulfanilamide compound. Dilute hydroalcoholic solutions of the compounds foam on shaking. Exposure of some of the salts to direct sunlight causes discoloration.

Hydroalcoholic solutions of the quaternary ammonium sulfa drugs are highly bactericidal and are more effective against Staph. aureus than E. typhosa. The bactericidal activity is equal and in some instances greater than the activity of the corresponding quaternary ammonium components.

SUMMARY

Quaternary ammonium salts of sulfanilamide derivatives were prepared from the sodium or silver salts of the sulfa drugs and quaternary ammonium halides. The physical and chemical properties of a number of such compounds are presented.

REFERENCES

(1) Domagk, G., *Deut. Med. Wochschr.*, **61**, 829(1935).
(2) Shelton, R. S., *et al.*, Abstracts 98th Meeting American Chemical Society, Sept., 1939; Abstracts 99th Meeting, American Chemical Society, April, 1940.
(3) Huyck, C. L., *Am. J. Pharm.*, **116**, 50(1944).
(4) Hoogerheide, J. C., *J. Bact.* **49**, 277(1945).

Qualitative Determination of Cocaine and Estradiol Benzoate*

By LAWRENCE S. MALOWAN

It was Guerbet (1) who first described a reaction for determining the presence of alkaloids such as atropine, cocaine, stovaine, etc., based on the presence of benzoic acid in the structural edifice of these substances. His method consisted of forming aminobenzoic acid, which, when diazotized, easily couples with β-naphthol, producing a red azo color. Guiges (2) carried out the transformation by evaporating the substance on the water bath with fuming nitric acid, reducing the product with a solution of stannous chloride, and then treating it with one per cent sodium nitrite.

Schorl (3) afterward thought that Guerbet's reaction did not give a good result in every case and proposed certain modifications which he used in the reaction for the determination of small quantities of benzoic acid. He was able to show, in particular, that metallic aluminum assists the reduction of the nitro product to the amine in the presence of stannous chloride. The reaction is so sensitive that, according to Schorl, 0.1 mg. of benzoic acid can be determined.

For this investigation Schorl's method, with some slight modifications, was applied to the detection of cocaine and on other benzoyl-containing drugs as, estradiol benzoate.[1]

Very few characteristic reactions exist for the determination of cocaine. Evaporation with sulfuric acid is merely an olfactory test, which is particularly unsatisfactory in presence of organic matter. In forensic chemistry the case often occurs in which the cocaine is adulterated with mannitol. Under such circumstances, the sulfuric acid test fails. The reaction with chromic acid is more satisfactory. It leaves room for errors, however, because quinine behaves like cocaine. The most specific test is the reaction

* From the Biochemical Laboratory, Inter. American University, Panama.

[1] Acknowledgment is made to the Schering Co., Bloomfield, N. J., for supplying a quantity of this substance.

with gold chloride or platinum chloride, the crystals formed being very characteristic. Nevertheless, in the presence of other organic or inorganic salts, the crystallization is not quite typical as in the case of the pure substance.

For the detection of cocaine, a method which would utilize the known reactions of benzoic acid, easily formed from cocaine by hydrolysis, was sought. The product resulting from the treatment of cocaine with hydrochloric acid with a neutral solution of ferric chloride was tested. The result was unsatisfactory. Then Schorl's method was employed for the determination of cocaine and estradiol benzoate, using 1,8-dihydroxy-naphthalene-3,6-disulfonic acid for the formation of the colored product.

The determination of the benzoyl part of estradiol benzoate, according to U. S. P. XII (4) is somewhat complicated, involving the hydrolytic separation of the benzoic acid from the estradiol complex which was found to be unnecessary. Carrying out a hydrolyzation of estradiol benzoate, according to U. S. P. XII, shows that the estradiol fraction does not give any reaction when submitted to Schorl's reaction employing chromotropic acid for formation of the colored compound. Only the benzoate remains in the filtrate. This experiment proved that the benzoate estradiol could be identified by submitting the substance to the reaction by the same method as in the case of cocaine.

EXPERIMENTAL

Five milligrams of the substance is dissolved in a few drops of hydrochloric acid 1:1 and evaporated on the water bath. When the residue is completely dry it is dissolved in a few drops of fuming nitric acid of specific gravity 1.5 and evaporated again. The residue is dissolved in a few drops of water and a few drops of 4 *N* potassium hydroxide; to it is added a small quantity of a solution of 10% stannous chloride in 4 *N* hydrochloric acid and a small piece of aluminum. This solution is left over ice for fifteen minutes. At the end of this time the aluminum is removed and a few drops of 1% sodium nitrite solution is added. In the meantime a solution of 0.5% 1,8-dihydroxy-naphthalene-3,6-disulfonic acid in about 10% ammonium hydroxide is prepared. The two solutions are mixed and an intense red-violet color is produced.

The reaction is specific for cocaine and is not given by atropine. Quinine, which is also used, according to experience in toxicology, for mixing with cocaine, does not give any reaction; neither does morphine. Estradiol benzoate gives this reaction as distinctly as cocaine.

Quantities as small as 2 mg. give a clear reaction following the ordinary method. The use of 1,8-dihydroxy-naphthalene-3,6-disulfonic acid is also recommended for the determination of benzoic acid, employing Schorl's technique.

REFERENCES

(1) Guerbet, *J. pharm. chim.*, **22**, 321(1920).
(2) Guiges, S., *Bull. sci. pharmacol.*, **35**, 292(1928).
(3) Schorl, N., *Pharm. Weekblad*, **77**, 425(1940).
(4) "Pharmacopœia of the United States XII," Mack Printing Company, Easton, Pa., 1942, p. 169.

The National Research Council's

INDUSTRIAL RESEARCH LABORATORIES IN THE UNITED STATES

The National Research Council has announced that it is now preparing the eighth edition of *Industrial Research Laboratories in the United States*. The seventh edition (1940) contained information concerning the industrial research laboratories of 2,264 companies and their subsidiaries. The Council is anxious to have the new edition of the Directory include all eligible organizations.

The term *research* for the purpose of the directory is construed as including investigations looking toward the improvement of products or the reduction of cost of manufacture as well as fundamental research and applied research. It does not apply to laboratories concerned only with commercial testing.

Research workers should inquire of their laboratory directors whether questionnaires have been received, as those reporting in 1940 will have received their printed statements for revision. If no questionnaire has been received, one will be sent upon request to the Library, National Research Council, Washington 25, D. C.

The directory is issued by the National Research Council with no expectation of profit. There is no charge for the inclusion of a statement regarding a laboratory and no obligation is incurred in supplying data.

Note on the Quantitative Determination of Menadione Sodium Bisulfite*

By KARL SCHOEN

A modification of the U. S. P. XII assay method for Menadione Sodium Bisulfite is proposed. Menadione is liberated from a water solution of menadione sodium bisulfite by the addition of sodium hydroxide, extracted with ether, and determined gravimetrically after evaporation of the solvent.

THE water-soluble addition product with anti-hemorrhagic activity which forms when menadione is shaken with concentrated sodium bisulfite solution (1, 2) is, according to Menotti (3), a simple addition product of the formula $C_{11}H_8O_2 \cdot NaHSO_3 \cdot 3H_2O$. Menotti was the first to describe the decomposition of the addition product with ammonium hydroxide to give free menadione, and the application of this reaction for the quantitative determination of menadione bisulfite. This method is described in the current edition of the U. S. Pharmacopœia for the assay of the product (4).

Menotti (3) has discussed also the color reaction of Craven (5) and of Pinder and Singer (6) for the quantitative determination of menadione bisulfite. These observations of Menotti on the reaction with ammonium hydroxide, and the color reaction have been confirmed by us. The results obtained by the colorimetric assay are, however, invariably low due to the presence of bisulfite. This causes a rapid fading of the color, especially when an excess of bisulfite or of sulfur dioxide is used to protect solutions from oxidation.

While Menotti has reported recoveries of 95 to 98 per cent of menadione from the addition product by decomposing with 10 per cent ammonia water, and his method has been adopted by the U. S. P., the method in our hands has some shortcomings. The free quinone is attacked by ammonia and strong alkalies, giving yellow to orange colored soluble products. The filtration also does not proceed very satisfactorily.

Therefore, we have devised a modified method, which has been used in our laboratory since April, 1943, with excellent results. Dilute sodium hydroxide solution is added, and the liberated menadione is extracted with ether and determined gravimetrically after cautious evaporation of the solvent. With this method we have obtained consistent results for over one year with an error of not more than 0.5 per cent.

* Received Jan. 22, 1945, from the Research Laboratory of Endo Products, Inc., Richmond Hill, N. Y.

EXPERIMENTAL

An aliquot of the solution containing about 100 mg. of menadione (approximately 200 mg. addition product), or a corresponding amount of the powder, is transferred to a separatory funnel. One drop of phenolphthalein indicator and just enough 0.1 *N* NaOH solution, to produce a permanent pink color, is added. The menadione is now extracted with five successive portions of ether, using 25, 20, 20, 15, and 15 ml.; after the second extraction more alkali is added (approximately 5 ml. 0.1 *N* NaOH) in order to insure complete decomposition. The combined ether extracts are filtered through a cotton plug into a tared beaker, the funnels are carefully washed with small quantities of ether, and the solvent is then evaporated by cautiously heating the beaker on a water bath with a fan blowing air over the mouth of the beaker. The last few milliliters of the ether are blown off without heating. The beaker is then brought to constant weight by keeping it overnight in a vacuum desiccator over phosphorus pentoxide.

Table I gives assay results of ampul solutions prepared in our laboratories.

TABLE I—ASSAY RESULTS OF AMPUL SOLUTIONS

Ampul Solution	Sample	Assay: Before Filling mg./1 Ml.	Assay: After Filling, Mg./1 Ml.
Menadione bisulfite, 2 mg. menadione per 1 ml.	1	2.03	2.05
	2	2.03	2.05
	3	2.08	2.09
	4	2.20[a]	2.00
		2.28	..
Special solution, 1 mg. menadione per 1 ml.	5	1.04	..

[a] Adjusted before filling.

REFERENCES

(1) Moore, M. B., *J. Am. Chem. Soc.*, **63**, 2049(1941).
(2) Baker, B. R., Davies, T. H., McElroy, L., and Carlson, G. H., *ibid.*, **64**, 1096(1942).
(3) Menotti, Amel R., *ibid.*, **65**, 1209(1943).
(4) "Pharmacopœia of the United States," Twelfth revision, FIRST SUPPLEMENT, Mack Printing Company, Easton, Pa., 1943, p. 43.
(5) Craven, R., *J. Chem. Soc.*, **1931**, 1605.
(6) Pinder, J. L., and Singer, J. H., *Analyst*, **65**, 7(1940).

BOOK REVIEWS

Inorganic Chemistry, by FRITZ EPHRAIM. Fourth English Edition edited by P. C. L. THORNE and E. R. ROBERTS. Nordeman Publishing Company, Inc., New York, 1944. xii + 921 pp. 23 x 15.5 cm. $8.75.

The first English edition of this book appeared in 1926 and was based upon the third German edition. The succeeding English editions have been modifications of the first translation and, hence, have not closely followed the later German revisions.

In the preface to this fourth English edition, the authors state that "The materials of inorganic chemistry are dealt with collectively rather than individually, with a consequent saving of space and of fatigue to the reader." Although this worthy objective has been efficiently realized, generalizations frequently so overshadow specific information that the latter is difficult to locate. At times, these generalizations are too inclusive; for instance, on page 543 the impression is given that each of the elements sulfur, selenium, and tellurium have trioxides which react with water to yield the corresponding -ic acids. Yet, on page 569, it is stated that tellurium trioxide is insoluble in water. Notwithstanding the limitations imposed by the emphasis on collective treatment of the elements by classes, this book has brought together, in a systematic way, an immense resevoir of current factual material in the field of inorganic chemistry. It is to be emphasized that the treatment is descriptive rather than theoretical.

The authors have upheld the reputation of English chemists for clarity of expression and the ability to present ideas in lucid and suggestive terminology. Illustrations of this are: "the pleiadic nature of many elements" and "an ancestral element" on page 28, "lead pleiad" on page 73, "innumerable nuances" on page 108, and "in the reguline condition" page 188.

Although this edition is very similar, in many respects, to the previous one, it has modernized interpretations in several instances. The reviewer is pleased to note, from page 843, that the compound known as sodium perborate is represented by the formula $NaBO_3 \cdot 3H_2O \cdot H_2O_2$. It is to be hoped that our official American pharmaceutical compendia will soon adopt this interpretation.

By using the "Photo Offset Reprint" process, the publishers have made more easily available this valuable reference at a moderate cost. This fact should extend its use and make the purchaser more tolerant of the misalignment of a letter here and there. There are comparatively few typographical errors. The formula for manganese dioxide, page 178, is a case in point.

The references which are cited are well selected and useful, but could be extended with profit. As is to be expected, they refer to English and German journals more frequently than to our American chemical literature.

This book is recommended as being a most useful and valuable adjunct for the reference shelf of those working in the field of inorganic chemistry.—ARTHUR E. JAMES.

Microbial Antagonisms and Antibiotic Substances, by SELMAN A. WAKSMAN. The Commonwealth Fund, New York, 1945. ix + 350 pages 23.5 x 15 cm. Price, $3.75.

That penicillin was discovered as early as the 1920's is well known, but it is easy to forget that the concept of microbial antagonism has been known for many decades. Since the earliest days of microbiology, antagonistic and symbiotic relationships among microbes have intrigued biologists.

Dr. Waksman has traced these microbial interrelationships, complex as they are, in a careful and scholarly account that dates back to Pasteur. The emphasis is distinctly that of a microbiologist but the chemical nature of the active principles of antibiotic agents is also discussed. To a chemist, it is of special interest to note the chemical relationship between the isolated antibiotic agents and known antiseptics, e.g., acridine and chloraphin and phenazine and pyocyanin.

Because of the timeliness of pencillin, the portion dealing with this agent is given the greatest attention. The section is factual and authoritative. It is unfortunate that for security reasons so little information can be made public. In a rapidly changing field, it is natural that many factors have changed since the writing of this book. For example, the statement "penicillin is not very stable" does not appear to be characteristic of the present-day product.

Many research workers are at present interested in improving ways of measuring antibiotic activity. It would seem logical that a test which takes into account position on the growth curve would add to the sensitivity of such tests. Professor Waksman's book calls attention to some studies that have been made on these relationships. Particularly pertinent is the "Bacterial spectrum" and the unique manner in which Marshall and Hrenoff have used the test to differentiate a disinfectant from a bacteriostatic agent. The factors influencing specificity are well brought out.

The antibiotic field is one which necessitates the closest cooperation between the chemist and the microbiologist (indeed many scientists are mutually involved). This work will serve as the leavening agent for such cooperative endeavors for some time to come.—MELVIN W. GREEN.